JOURNAL OF BACTERIOLOGY, Apr. 1982, p. 319–326
0021-9193/82/040319-08$02.00/0

Vol. 150, No. 1

Respiratory Physiology and Energy Conservation Efficiency of *Campylobacter jejuni*

PAUL S. HOFFMAN†* AND TOM G. GOODMAN

Microbiology Section, Department of Biology, Memphis State University, Memphis, Tennessee 38152

Received 30 July 1981/Accepted 21 November 1981

A study of the electron transport chain of the human intestinal pathogen *Campylobacter jejuni* revealed a rich complement of *b*- and *c*-type cytochromes. Two *c*-type cytochromes were partially purified: one, possibly an oxidase, bound carbon monoxide whereas the other, of high potential was unreactive with carbon monoxide. Respiratory activities determined with membrane vesicles were 50- to 100-fold higher with formate and hydrogen than with succinate, lactate, malate, or NADH as substrates. Evidence for three terminal respiratory components was obtained from respiratory kinetic studies employing cyanide, and the following K_i values for cyanide were determined from Dixon plots: ascorbate + reduced *N,N,N',N'*-tetramethyl-*p*-phenylenediamine, K_i = 3.5 μM; malate, K_i = 55 μM; and hydrogen, K_i = 4.5 mM. Two oxidases (K_i = 90 μM, 4.5 mM) participated in the oxidation of succinate, lactate, and formate. Except with formate, 37 μM HQNO inhibited respiration by approximately 50%. Carbon monoxide had little inhibitory effect on respiration except under low oxygen tension (<10% air saturation). The stoichiometry of respiratory-driven proton translocation (H^+/O) determined with whole cells was approximately 2 for all substrates examined except hydrogen (H^+/O = 3.7) and formate (H^+/O = 2.5). The higher stoichiometries observed with hydrogen and formate are consistent with their respective dehydrogenase being located on the periplasmic face of the cytoplasmic membrane. The results of this study suggest that the oxidation of hydrogen and formate probably serves as the major sources of energy for growth.

Campylobacter jejuni Veron (32) (*Campylobacter fetus* subsp. *jejuni*) is now recognized as a common cause of human enteritis (2, 19, 29). This unusual vibroid organism is microaerophilic, fails to catabolize sugars, and has a strictly respiratory form of metabolism. Isolation of *C. jejuni* from clinical specimens usually requires special media and incubation under decreased oxygen tension (2, 19, 30). Strains of *C. fetus* appear to be acutely sensitive to the effects of low levels of hydrogen peroxide and superoxide radicals, despite the presence of catalase and superoxide dismutase inside the cell (10, 11). Moreover, addition of superoxide dismutase and catalase to the brucella-based medium, used to culture *C. fetus*, permits many of the strains to grow aerobically. Enhanced aerotolerance could also be achieved by supplementing medium with a combination of ferrous sulfate, sodium metabisulfite, and sodium pyruvate (FBP broth; 6). These substrates were further shown to spontaneously decompose hydrogen peroxide and superoxide radicals.

Although it is clear that reduced forms of oxygen exert a toxic effect on *C. jejuni*, particularly at higher oxygen tensions, the mechanism(s) of the toxicity remains unresolved. The oxidative metabolism of *C. jejuni* may even contribute to the oxygen toxicity. In this regard Niekus et al. (26) reported that in *C. sputorum* subsp. *bubulus*, superoxide and hydrogen peroxide were generated by the respiratory metabolism. The observation is particularly significant considering that *C. sputorum* lacks catalase. In *C. jejuni*, as well as in *C. sputorum*, hydrogen peroxide is generated during oxidation of formate (11, 24). Further, *C. jejuni* grows poorly even under microaerophilic conditions on media supplemented with formate (10, 11).

Reduced forms of oxygen may exert a direct effect on specific respiratory components, such as iron sulfur proteins or terminal oxidases (18, 23, 34). Many types of terminal oxidases, particularly those of low potential, autooxidize when exposed to high partial pressures of oxygen (18, 33). Harvey and Lascelles (8) reported that some of the *c*- and *b*-type cytochromes present in the membrane of *C. fetus* subsp. *intestinalis* were probably of low potential. One potential oxidase of the cytochrome *c*-type was reported in this study based on its reactivity with carbon monox-

† Present address: Department of Microbiology, University of Georgia, Athens, GA 30602.

ide. It is likely that this *c*-type oxidase is of low potential and may be synonomous with the cytochrome *c* reductase reported in an earlier study (10).

This study was initiated to determine the organization and function of the respiratory chain and its possible role in the microaerophilic nature of *C. jejuni*. To our knowledge, there have been no studies into the energy conservation efficiency for this species. Further, this study addresses the role of hydrogenase and formate dehydrogenase in the respiratory physiology and energy conservation efficiency.

MATERIALS AND METHODS

Organism and culture conditions. *C. jejuni* strain H840 (ATCC 29428) was maintained in brucella semisolid medium (0.15% agar) at 37°C and transferred weekly. Starter cultures for batch culture were grown in 250-ml flasks containing 100 ml of brucella broth (Difco Laboratories). The stoppered flasks were evacuated and filled with nitrogen to achieve an atmosphere of approximately 6% oxygen and incubated statically overnight at 37°C. The flasks were then placed on a rotary shaker and incubated aerobically for 24 h. Three 4-liter fermentors (New Brunswick Scientific Co.) containing 3.5 liters of brucella broth were inoculated with starter culture and incubated at 37°C with stirring (140 rpm), but no aeration, for about 6 h. Low aeration was then initiated until late-log phase, when the aeration was increased to 0.25 liters per min. This procedure yielded approximately 1.0 g (wet weight) of cells per liter of culture.

Collection of cells and preparation of membrane vesicles. Cells were harvested during late-log phase by centrifugation at 6,000 × *g* for 10 min at 4°C. The cell pellets were pooled, suspended in phosphate-salts buffer (pH 7.3), and washed once. The phosphate-salts buffer contained per liter: NaCl, 4.0 g; KCl, 2.0 g; K_2HPO_4, 1.0 g; and $MgSO_4 \cdot 7H_2O$, 1.8 g. For proton translocation studies, cells were suspended in 150 mM KCl. Cell pastes were stored at −20°C when not immediately used.

Membrane vesicles were prepard by suspending cell pastes in phosphate-salts buffer at pH 7.3 (2.2 ml per g [wet weight]) and disrupted by two passages through a French pressure cell at 4°C. The crude extract was then centrifuged at 29,000 × *g* for 15 min to remove cell debris, followed by centrifugation at 100,000 × *g* for 90 min to pellet the membrane vesicles. The membrane pellet was suspended in the 20 mM phosphate buffer to approximately 40 mg of protein per ml and used immediately.

Solubilization of cytochrome components. Crude cell-free extracts (prepared from 35 g [wet weight] of cells) containing 0.5% (wt/vol) Triton X-100 were adsorbed onto a DE-52 column (3 by 9 cm) equilibrated in 50 mM Tris-hydrochloride at pH 8 and eluted with a linear gradient of sodium chloride (0.05 to 0.8 M). Fractions were dialyzed against three one-liter changes of 25 mM Tris-hydrochloride (pH 8), readsorbed onto DE-52, and similarly eluted. Cytochrome fractions were determined optically with a Gilford 250 scanning spectrophotometer. Those fractions containing cytochrome *c* and the carbon monoxide-binding cytochrome *c'* were concentrated by ultrafiltration (PM-10 membrane; Amicon Corp.), and partial purification was estimated from optical spectra according to Campbell et al. (3).

Cytochrome spectroscopy. Cytochrome scans were performed with a Gilford 250 scanning spectrophotometer, or for low-temperature spectra (77°K) an Aminco DW-2 (American Instruments Co.) was used. Dithionite-reduced minus air or potassium ferricyanide-oxidized difference spectra were determined with membrane vesicles, and the types of cytochromes and concentrations were estimated by using extinction coefficients and wavelength pairs reported by Jones and Redfearn (17). Absolute spectra (reduced and oxidized) were determined for the partially purified cytochromes *c* and *c'* as well as the heme type by extracting the hemes in alkaline pyridine (13).

Oxygen consumption. Oxygen consumption was measured with an oxygen electrode (Yellow Springs Instruments Co.) inserted into a Gilson water-jacketed chamber. A circulating water bath was used to maintain constant temperature of 37°C. Membrane vesicles were suspended in the 1.35-ml chamber containing 50 mM potassium phosphate (pH 7.0) and allowed to equilibrate for 2 min. Various substrates were added through a glass capillary bore stopper to initiate the reaction. The amount of dissolved oxygen in the chamber at 37°C was calculated to be 210 μM from values obtained from Umbreit et al. (31). The following substrates were used: 1 mM NADH, 5 mM malate, 20 mM succinate, 5 mM lactate, 5 mM formate, and 4 mM ascorbate + 2 mM *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD). Oxidation of hydrogen was determined by injecting 200 μl of hydrogen-saturated 20 mM phosphate buffer into the Gilson chamber and measuring the steady-state oxygen consumption which followed a rapid decrease in the partial pressure of oxygen resulting from the addition. Respiration rates were expressed in nanomoles of oxygen consumed per minute per milligram of protein. Protein was determined by the biuret method with bovine serum albumin as a standard (7).

Localization of hydrogenase and formate dehydrogenase was determined by the method of Odom and Peck (27), using methyl viologen and benzyl viologen. Both whole cells and cell extracts were used, and total activity was determined with the permeant dye methylene blue.

Kinetic studies employing cyanide. The kinetics of cyanide-inhibited respiration were used to determine the number of oxidases participating in the oxidation of particular substrates (9, 14). For these experiments, the cyanide concentration was varied from 10^{-7} to 10^{-2} M, and the respiration rate at each cyanide concentration was recorded for each substrate used. Kinetic data were obtained with succinate, formate, malate, lactate, and hydrogen as substrates, and the results were plotted as percent activity (control without cyanide = 100%) versus cyanide concentration. Monophasic plots suggested the presence of one oxidase, whereas biphasic plots were considered indicative of the presence of more than one oxidase. Dixon plots ($1/v$ versus cyanide concentration) were used to determine K_i values for cyanide for each oxidase. The K_i values reported were obtained under conditions of excess substrate and oxygen concentration.

Effects of carbon monoxide and HQNO on respiration. The inhibitor 2-heptyl-4-hydroxyquinoline-*N*-oxide was used to determine the entry point of electrons into the electron transport chain. The reaction was carried out in the Gilson chamber at 37°C by adding membranes and HQNO (37 μM) and allowed to equilibrate for 2 min before the addition of substrate. The effect of carbon monoxide on respiration with various substrates was also determined. The desired ratio of CO to O_2 was obtained by mixing portions of CO-, O_2-, and N_2-saturated solutions of 20 mM potassium phosphate buffer. Membrane vesicles were then added, and the chamber was closed with a ground glass capillary stopper. After equilibration (5 min), substrates were added. Inhibition by CO was reported as percent control in the absence of CO.

Proton translocation. The stoichiometry of respiratory-driven proton translocation was determined by the general method of Mitchell and Moyle (22) as modified by Hoffman et al. (12). In this study, we used an 8-ml water-jacketed chamber, into which an oxygen electrode was inserted from one side and a pH electrode (Markson) was inserted through an adjustable top. The reaction mixture used was a modification of that of Downs and Jones (5) and contained 150 mM potassium chloride, 100 mM potassium isothiocyanate, 500 μM Tris-hydrochloride (pH 7.0), and 40 μg of carbonic anhydrase. Substrates were added at 1.25 to 2 mM for the potassium salts of formate, succinate, lactate, and malate. For potassium ascorbate and TMPD, the pH was adjusted to 7.0 before the addition of whole cells. Hydrogen was added by sparging the gas into the reaction mixture. Whole cells (1 to 3 m/ml [dry weight]) and carbonic anhydrase were added to the chamber after the mixture had been rendered anaerobic by sparging with nitrogen gas (passed through water) or with hydrogen. Residual oxygen was removed from the chamber by respiration. A modified Fisher Accumet 525 pH meter was used to monitor changes in pH, and adjustments in pH were made by the addition of either 50 mM KOH or HCl to achieve a starting pH of 7.0. The mV output of the pH meter was amplified with an analog preamplifier, and traces of pH changes were recorded with a Cole-Palmer recorder. Air-saturated 150 mM KCl (470 nanoatoms 0 at 25°C) was added to the 8-ml chamber through a portal in the adjustable top. Volumes of air-saturated KCl injected into the chamber were in the range of 10 to 30 μl. The system was calibrated by additions of 1 μl of anaerobic 50 mM HCl (6.25 nmol of H^+ per ml of reaction mixture). Calculation of the H^+/O ratio was determined from the ΔpH as described by Mitchell and Moyle (22) and corrected for collapse of the pH gradient by the method of Scholes and Mitchell (28). Values reported represent the mean of at least five determinations for each substrate and for several batches of freshly grown cells. As controls, H^+/O quotients were determined for *Azotobacter vinelandii* and for rat liver mitochondria. The effect of the uncoupling agent carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP) on the collapse of the proton gradient was also determined.

RESULTS

Cytochrome studies. Room temperature difference spectra of membrane vesicles from cells grown under microaerophilic conditions revealed cytochromes of the *b*- and *c*-type. Cytochrome *c* was the most abundant cytochrome, and some of the cytochrome appeared to be soluble. The concentration of membrane-bound cytochrome *c* was 1.53 nmol per mg of protein (wavelength pair, 552 and 545 nm). Cytochrome *b* appeared to be membrane bound and was present at 1.3 nmol per mg of protein (559 and 575 nm). Low-temperature difference spectra (77°K) indicated a splitting in the 556- to 559-nm region, suggesting the presence of two *b*-type cytochromes (Fig. 1). We could find no spectral evidence for cytochromes of the *o*-, *a*-, or *d*-type.

A carbon monoxide-binding *c*-type cytochrome was detected in CO-reduced minus reduced difference spectra (spectra not shown). To clearly distinguish it from cytochrome *o* (558 nm), the cytochrome was partially purified. After solubilization in Triton X-100 and two passages through a DE-52 column, a partial purification of three- to fivefold was obtained (0.4 nmol per mg of protein). The cytochrome was olive in color and had reduced absolute absorption maxima at 414, 523, and 552 nm. When CO was bubbled into the cuvette, the absorption maximum at 552 nm decreased dramatically (Fig. 2a). The decrease in extinction at 552 nm is characteristic for CO-binding cytochromes and is due to a higher oxidation state resulting from the CO-heme complex. To determine whether the cytochrome was of high or low potential, ascorbate plus the high-potential electron donor TMPD were used to reduce the cytochrome. The CO-binding cytochrome *c* was not reduced by reduced TMPD, but this may have been due to a lack of specificity. Absolute spectra of the

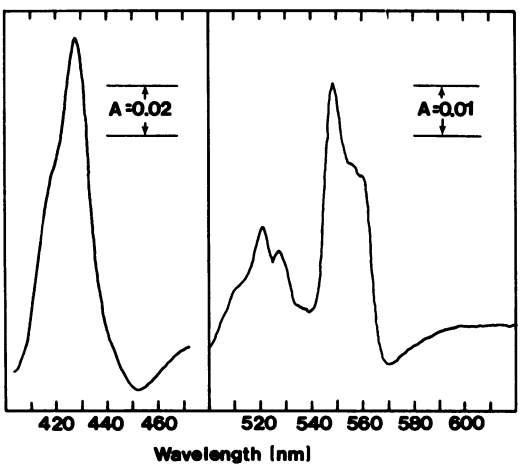

FIG. 1. Low-temperature (77°K) difference spectrum of membrane vesicles of *C. fetus* subsp. *jejuni*. The protein concentration was 3.3 mg/ml.

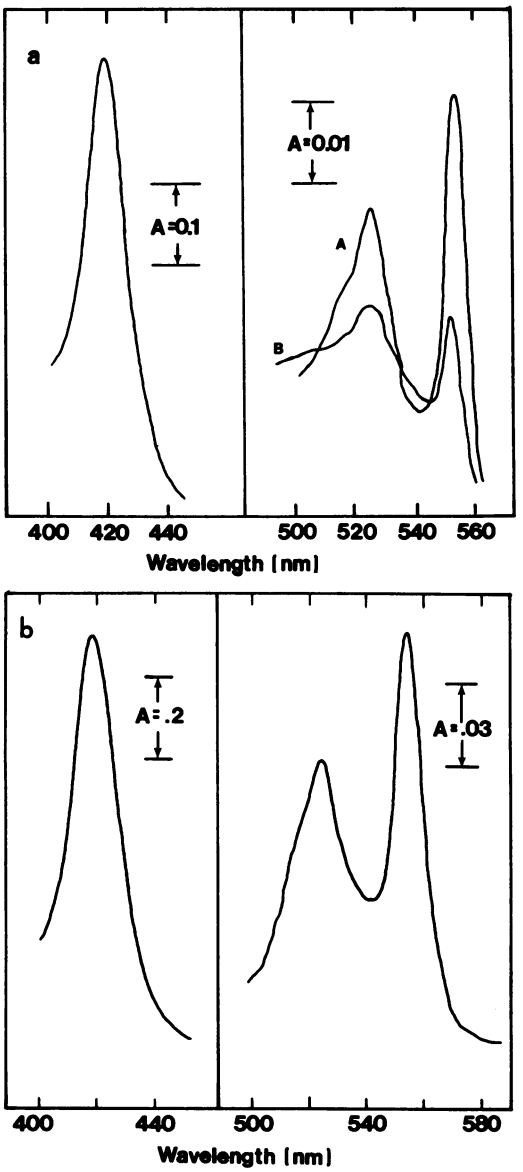

FIG. 2. Absolute cytochrome spectra of cytochromes *c'* and *c*. (a) Dithionite-reduced (A) and CO + dithionite-reduced (B) absolute spectra of partially purified cytochrome *c'*. Water was used in the reference cuvette, and the protein concentration was 1.4 mg/ml. (b) Reduced absolute spectrum of the partially purified, high-potential cytochrome *c*. The protein concentration was 0.7 mg/ml.

cytochrome extracted in alkaline pyridine exhibited a characteristic spectrum of a *c*-type cytochrome with an α-absorption maximum at 550 nm. In accordance with suggested nomenclature for *c*-type cytochromes (1), the CO-binding cytochrome will be referred to as cytochrome *c'* to distinguish it from other *c*-type cytochromes.

A second *c*-type cytochrome, which did not react with carbon monoxide, was partially purified (threefold purification). This red cytochrome had reduced absolute absorption maxima at 414, 525, and 554 nm (Fig. 2b). Ascorbate plus reduced TMPD readily reduced this cytochrome, indicating that it is a high-potential *c*-cytochrome, and extraction of the heme in alkaline pyridine revealed a characteristic spectrum of cytochrome *c*.

Respiration rates and inhibitor studies. The respiratory activities determined for membrane vesicles are reported in Table 1. The membrane-bound dehydrogenases having the highest activities were formate dehydrogenase and hydrogenase. These activities were 50- to 100-times higher than those observed for succinate, malate, lactate, and NADH dehydrogenases. The NADH dehydrogenase activity was very low (5 nmol per min per mg of protein).

Hydrogenase and formate dehydrogenase were located on the external side of the cytoplasmic membrane, as evidenced by the reduction of viologen dyes. Total hydrogenase activity was similar for whole cells and for cell extracts (133 nmol per min per mg of protein), whereas lactate oxidation was 60% greater in cell extracts. Whole cells rapidly reduced the permeant dye methylene blue with lactate, formate, or hydrogen as substrates. All three enzymatic activities remained in the membrane fraction after ultracentrifugation.

Kinetic studies of cyanide-inhibited respiration were used to determine the number of potential oxidases in the respiratory chain. When the percent respiratory activity was plotted against cyanide concentration for substrates such as succinate, lactate, and formate, biphasic plots were obtained (Fig. 3). Two oxidases having K_i values for cyanide of approximately 90 μM and 4.5 mM were obtained from Dixon plots (data not shown). However, with malate as substrate (Fig. 4), only one oxidase was detect-

TABLE 1. Respiration rates for membrane vesicles of *C. jejuni*

Substrate[a]	Respiration rate[b]
NADH	5.3 ± 1.5
Formate	226.8 ± 9.3
Lactate	11.0 ± 2.4
Succinate	15.7 ± 5.0
Malate	10.9 ± 0.68
Hydrogen	219.6 ± 10.4
Ascorbate + TMPD	60 ± 8.0

[a] Respiration rates were determined at 37°C with substrates in excess as described in the text.

[b] Respiration rates are in nanomoles of oxygen consumed per minute per milligram of protein.

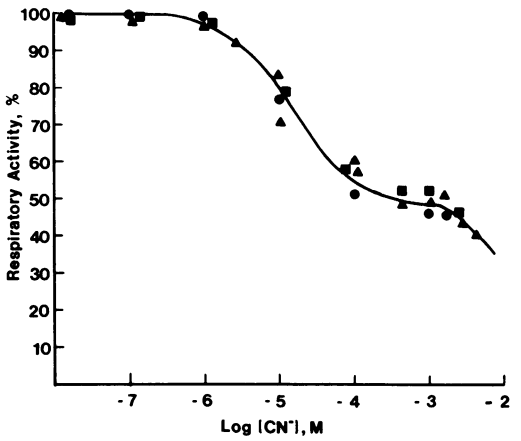

FIG. 3. Effect of cyanide concentration on the respiration rate of membrane vesicles. The percent respiratory activity was normalized to a control without cyanide. The 100% respiration rates are as reported in Table 1. The following substrates were used: succinate (●), lactate (▲), and formate (■). From Dixon plots, K_i values for cyanide of 90 μM and 4.5 mM were obtained.

ed, having a K_i for cyanide of 55 μM. This oxidase appeared to be the same as the first oxidase (K_i = 90 μM) detected for succinate, lactate, and formate. When ascorbate and reduced TMPD were used as substrate, an oxidase of high cyanide sensitivity was detected having a K_i of 3.5 μM (Fig. 4). This oxidase was not observed with any of the physiological substrates used in this study. Hydrogen appears to be oxidized through a cyanide-resistant oxidase having a K_i value of 4.5 mM. No effort was made to assign these K_i values to any particular oxidase.

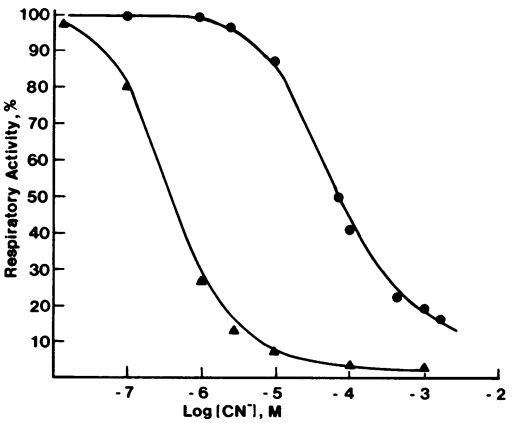

FIG. 4. Effect of cyanide concentration on the respiration rate for membrane vesicles with ascorbate + TMPD (▲) and malate (●) as substrates. The 100% respiration rates are as reported in Table 1. From Dixon plots, K_i values for cyanide of 3.5 μM (ascorbate + TMPD) and 55 μM (malate) were obtained.

The respiratory chain of *C. jejuni* was only partially sensitive to the inhibitory effects of HQNO. This inhibitor is believed to act at the quinone side of cytochrome *b* and is commonly used to establish entry points for substrates into the electron transport chain (18). In this regard, HQNO had no effect on formate oxidation, whereas oxidation of succinate, malate, and lactate were inhibited 50 to 60% by HQNO (37 μM). Hydrogen oxidation was similarly inhibited by HQNO. Increasing the concentration of HQNO appeared to have no significant effect. Oxidation of ascorbate plus reduced TMPD was not affected by HQNO.

The oxidases were also generally insensitive to inhibition by carbon monoxide. At 30% air saturation, CO inhibited respiration approximately 25% for the substrates succinate, formate, and hydrogen. However, when the oxygen tension was lowered to 10% saturation without increasing the CO concentration (differences corrected with nitrogen-saturated buffer), the inhibition increased to between 50 and 60%. Interestingly, CO had no inhibitory effect on oxidation of ascorbate plus reduced TMPD, suggesting that TMPD oxidation might be mediated by a component other than an oxidase.

Stoichiometry of proton translocation. The results from H^+/O studies are presented in Table 2. These studies were performed with whole cells, and the maximum H^+/O quotient of 3.7 was obtained with hydrogen as substrate. An H^+/O ratio of approximately 2 was obtained with succinate, malate, and lactate. With formate an H^+/O ratio of 2.5 was obtained. The higher stoichiometry with formate is probably due to liberation of an additional proton during formate oxidation (4, 21). The uncoupling agent FCCP greatly accelerated the collapse of the proton gradient. Proton stoichiometries were similar for experiments performed at either 25 or 37°C, and the H^+/O quotients obtained with

TABLE 2. H^+/O ratios for whole cells of *C. jejuni*

Substrate	H^+/O[a]
Formate	2.50 ± .32
Hydrogen	3.72 ± .13
Malate	2.03 ± .21
Succinate	2.01 ± .13
Lactate	2.12 ± .17
Sulfite	1.58 ± .21
Asc + TMPD	0
Endogenous	0

[a] H/O quotients represent the mean and standard deviation for at least five determinations.

whole cells of *A. vinelandii* and with rat liver mitochondria were consistent with published values (12, 22).

Substrates leading to the generation of NADH were oxidized so poorly that determinations of H^+/O ratios could not be made. Oxidation of ascorbate plus TMPD did not produce a proton pulse, suggesting that no coupling site exists between cytochrome *c* and the terminal oxidases. Interestingly, sulfite, which had been previously reported to enhance the aerotolerance of strain H840, when oxidized produced a proton pulse (H^+/O ratio = 1.6). Sulfite may therefore serve as an energy source.

DISCUSSION

The respiratory physiology of the human intestinal pathogen *C. jejuni* is not only complex, but also appears to be uniquely suited for low-oxygen environments, such as the intestinal tract. *Campylobacter* species in general contain high concentrations of *c*- and *b*-type cytochromes (8, 11). Harvey and Lascelles (8) suggested that some of the *c*-type cytochromes are of low redox potential and participate in the oxidation of physiological substrates, whereas cytochrome *c* of higher potential mediates the oxidation of reduced TMPD. We partially purified one cytochrome *c* which was of high redox potential. This *c* cytochrome is probably not an oxidase since it failed to bind CO. Moreover, the terminal oxidase for this branch may not be a *c*-type cytochrome. The cytochrome *c* which might be an oxidase on the basis of its reactivity with CO may be of low potential since it failed to oxidize reduced TMPD. This strain of *C. jejuni* has cytochrome *c* reductase activity (10), and it is likely that the low potential cytochrome *c'* reduces oxidized horse heart cytochrome *c* (100 mV). If cytochrome *c'* is indeed a terminal oxidase, it is probably associated with a *b*-type cytochrome of lower redox potential. The oxidase may be involved in the oxygen toxicity, and this possibility is being further examined.

Although cytochrome *c'* appears to be the only potential oxidase, as determined by optical spectroscopy, evidence for multiple oxidases has been obtained with cyanide. Three K_i values for cyanide were obtained, but it has not been possible to assign these to any particular oxidase. Inhibition studies with cyanide also suggest that the respiratory chain is branched since the oxidation of succinate, lactate, and formate are mediated by two oxidases (K_i 90 μM, 4.5 mM). Interestingly, results with HQNO suggest that electrons from formate oxidation enter the respiratory chain at a different point than do those from succinate and lactate. In some bacteria, formate dehydrogenase is associated with a *b*-type cytochrome (8). Two *b*-type cytochromes were resolved in low-temperature difference spectra, but their location and function were not determined.

The entry point of electrons from the oxidation of malate, hydrogen, formate, and ascorbate plus TMPD presents several problems. In the case of malate, oxidation is mediated by a single oxidase (K_i = 55 μM), which is similar to the first oxidase observed with succinate and formate. Similarly, with hydrogen, the cyanide-insensitive oxidase mediates the oxidation, but it also mediates the oxidation of succinate and formate. Moreover, the oxidation of hydrogen, malate, and succinate are partially sensitive to HQNO, whereas formate oxidation is not. Interestingly, Niekus et al. (25) have reported that in *C. sputorum* subsp. *bubulus*, HQNO partially inhibited formate oxidation (32%) only at low dissolved oxygen tensions (5.1 μM O_2). A similar inhibition was not apparent with *C. jejuni*. Possibly the location of these enzymes in the membrane might be considered in explaining these results. Both formate dehydrogenase and hydrogenase appear to be located on the external face of the cytoplasmic membrane, whereas the dehydrogenases for succinate, malate, and lactate are located on the inner face. The results with HQNO would suggest that formate dehydrogenase and hydrogenase are possibly located around different redox centers. Since inhibition by HQNO was less than 60%, HQNO must be considered a poor inhibitor unless alternate routes of electron flow are occurring in the respiratory chain.

In regard to the ascorbate TMPD-oxidase, no physiological substrates were found to have a similar K_i value for cyanide; the role of this component as an oxidase is questionable. This possibility is further supported by the inability of CO to inhibit TMPD oxidation.

Energy conservation efficiency. A low-energy conservation efficiency was observed in *C. jejuni* grown under microaerophilic conditions. The energy conservation efficiency is lower than that reported for other aerobic bacteria containing cytochrome *c* (15, 16). Similar results have been reported by DeVries et al. for *C. sputorum* subsp. *bubulus* (4). Essentially, with all substrates tested except hydrogen and formate, one mole of ATP is apparently generated per gram atom of oxygen consumed. The location of the proton-translocating site is unresolved, but is probably located in the quinone-cytochrome *b* region of the electron transport chain. There appears to be no proton-translocating site between cytochrome *c* (high potential) and the terminal oxidase. However, we could not rule out the possibility that NADH dehydrogenase is a proton pump since substrates expected to

generate NADH were oxidized too slowly for accurate measurement of a proton pulse.

Hydrogen and formate were the most energy-efficient substrates, and the higher stoichiometries observed are consistent with a periplasmic location of hydrogenase and formate dehydrogenase. Similar results have been reported by Kröger et al. (21) for *Vibrio succinogenes*. Formate oxidation liberates $CO_2 + H^+$, and the two electrons generated yield an additional $2H^+$ via the respiratory chain (4, 21). The high stoichiometry with hydrogen also reflects its oxidation in the periplasmic space ($2H^+ + 2e^-$) with an additional $2H^+$ generated via the respiratory chain. Thus, proton stoichiometries approaching 3 with formate and 4 with hydrogen would be expected. Similar conclusions have been reported for *C. sputorum* subsp. *bubulus* and *V. succinogenes* (4, 21). The slightly lower H^+/O value obtained in this study with formate might be due to a slight uncoupling effect reported for formate (4).

To our knowledge, hydrogenase has not been documented in *C. jejuni*. Although Kiggins and Plastridge (20) reported hydrogenase activity in 4 of 32 strains of *C. fetus* isolated from cattle, nomenclatural problems prevent any clear conclusions as to species. We detected hydrogenase activity in all *C. jejuni* strains in our laboratory and are currently surveying other *Campylobacter* strains for hydrogenase activity.

ACKNOWLEDGMENTS

We thank D. V. DerVartanian, Department of Biochemistry, University of Georgia for use of the Aminco DW-2 spectrophotometer.

This study was partially supported by a grant from the Sponsored Program Office, Memphis State University.

LITERATURE CITED

1. **Bartsch, R. G.** 1968. Bacterial cytochromes. Annu. Rev. Microbiol. **22**:181–200.
2. **Blaser, J. J., I. D. Berkowitz, F. M. LaForce, J. Cravens, L. B. Reller, and W.-L. L. Wang.** 1979. *Campylobacter* enteritis: clinical and epidemiological features. Ann. Intern. Med. **91**:179–185.
3. **Campbell, W. H., W. H. Orme-Johnson, and R. H. Burris.** 1973. A comparison of the physical and chemical properties of four cytochromes *c* from *Azotobacter vinelandii*. Biochem. J. **135**:617–630.
4. **DeVries, W., H. G. D. Niekus, M. Boellaard, and A. H. Stouthamer.** 1980. Growth yields and energy generation by *Campylobacter sputorum* subspecies *bubulus* during growth in continuous culture with different hydrogen acceptors. Arch. Microbiol. **124**:221–227.
5. **Downs, A. J., and C. W. Jones.** 1975. Respiration-linked proton translocation in *Azotobacter vinelandii*. FEBS Lett. **60**:42–46.
6. **George, H. A., P. S. Hoffman, N. R. Krieg, and R. M. Smibert.** 1978. Development of an improved culture medium for *Campylobacter fetus*. J. Clin. Microbiol. **8**:36–41.
7. **Gornall, A. G., G. J. Bardawill, and M. M. David.** 1949. Determination of serum proteins by means of the biuret reaction. J. Biol. Chem. **177**:751–766.
8. **Harvey, S., and J. Lascelles.** 1980. Respiratory systems and cytochromes in *Campylobacter fetus* subsp. *intestinalis*. J. Bacteriol. **144**:917–922.
9. **Henry, M. F., and P. M. Vignais.** 1979. Induction by cyanide of cytochrome *d* in the plasma membrane of *Paracoccus denitrificans*. FEBS. Lett. **10**:41–46.
10. **Hoffman, P. S., H. A. George, N. R. Krieg, and R. M. Smibert.** 1979. Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni*. II. Role of exogenous superoxide anions and hydrogen peroxide. Can. J. Microbiol. **25**:8–16.
11. **Hoffman, P. S., N. R. Krieg, and R. M. Smibert.** 1979. Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni*. I. Physiological aspects of enhanced aerotolerance. Can. J. Microbiol. **25**:1–7.
12. **Hoffman, P. S., T. V. Morgan, and D. V. DerVartanian.** 1979. Respiratory-chain characteristics of mutants of *Azotobacter vinelandii* negative to tetramethyl-p-phenylenediamine oxidase. Eur. J. Biochem. **100**:19–27.
13. **Hoffman, P. S., T. V. Morgan, and D. V. DerVartanian.** 1980. Respiratory properties of cytochrome-c-deficient mutants of *Azotobacter vinelandii*. Eur. J. Biochem. **110**:349–354.
14. **Jones, C. W.** 1973. The inhibition of *Azotobacter vinelandii* terminal oxidases by cyanide. FEBS Lett. **36**:347–350.
15. **Jones, C. W., J. M. Brice, A. J. Downs, and J. W. Drozd.** 1975. Bacterial respiration-linked proton translocation and its relationship to respiratory-chain composition. Eur. J. Biochem. **52**:265–271.
16. **Jones, C. W., J. M. Brice, and C. Edwards.** 1977. The effects of respiratory chain composition on the growth efficiencies of aerobic bacteria. Arch. Microbiol. **115**:85–93.
17. **Jones, C. W., and E. R. Redfearn.** 1966. Electron transport in *Azotobacter vinelandii*. Biochim. Biophys. Acta **113**:467–481.
18. **Jurtshuk, P., Jr., T. J. Mueller, and W. C. Acord.** 1975. Bacterial terminal oxidases. Crit. Rev. Microbiol. **3**:399–468.
19. **Kaplan, R. L.** 1980. *Campylobacter*, p. 235–241. *In* E. J. Lennette (ed.), Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington D.C.
20. **Kiggins, E. M., and W. N. Plastridge.** 1956. Effect of gaseous environment on growth and catalase content of *Vibrio fetus* cultures of bovine origin. J. Bacteriol. **72**:397–400.
21. **Kröger, A., E. Dorrer, and E. Winkler.** 1980. The orientation of the substrate sites of formate dehydrogenase and fumarate reductase in the membrane of *Vibrio succinogenes*. Biochim. Biophys. Acta **589**:118–136.
22. **Mitchell, P., and J. Moyle.** 1967. Respiratory-driven proton translocation in rat liver mitochondria. Biochem. J. **105**:1147–1162.
23. **Morris, J. G.** 1975. The physiology of obligate anaerobiosis. Adv. Microb. Physiol. **12**:169–246.
24. **Niekus, H. G. D., E. van Doorn, W. DeVries, and A. H. Stouthamer.** 1980. Aerobic growth of *Campylobacter sputorum* subspecies *bubulus* with formate. J. Gen. Microbiol. **118**:419–428.
25. **Niekus, H. G. D., E. van Doorn, and A. H. Stouthamer.** 1980. Oxygen consumption by *Campylobacter sputorum* subspecies *bubulus* with formate as substrate. Arch. Microbiol. **127**:137–143.
26. **Niekus, H. G. D., C. H. Wouters, W. DeVries, and A. H. Stouthamer.** 1978. Superoxide dismutase and hydrogen peroxide formation in *Campylobacter sputorum* subspecies *bubulus*. Arch. Microbiol. **119**:37–42.
27. **Odom, J. M., and H. D. Peck, Jr.** 1981. Localization of dehydrogenases, reductases, and electron transfer components in the sulfate-reducing bacterium *Desulfovibrio gigas*. J. Bacteriol. **147**:161–169.
28. **Scholes, P., and P. Mitchell.** 1970. Respiration-driven proton translocation in *Micrococcus denitrificans*. J. Bioenerg. **1**:309–323.
29. **Skirrow, M. B.** 1977. *Campylobacter* enteritis: a "new" disease. Br. Med. J. **2**:9–11.
30. **Smibert, R. M.** 1978. The genus *Campylobacter*. Annu.

Rev. Microbiol. 32:673–709.
31. **Umbreit, W. W., R. H. Burris, and J. F. Stauffer.** 1974. Manometric techniques, p. 5. Burgess Publishing Co., Minneapolis.
32. **Veron, M., and R. Chatelain.** 1973. Taxonomic study of the genus *Campylobacter* Sebald and Veron and designation of the neotype strain for the type species *Campylobacter fetus* (Smith and Taylor) Sebald and Veron. Int. J. Syst. Bacteriol. 23:122–134.
33. **Yang, T. Y., and P. Jurtshuk, Jr.** 1978. Studies on the red oxidase (cytochrome o) of *Azotobacter vinelandii*. Biochem. Biophys. Res. Commun. 81:1032–1039.
34. **Yoch, D. C., and R. P. Carithers.** 1979. Bacterial iron-sulfur proteins. Microbiol. Rev. 43:384–421.